COMPOSITIONS AND METHODS FOR TREATMENT OF CHEMICAL WARFARE AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Ser. No. 62/803,006, filed on Feb. 8, 2019, U.S. Ser. No. 62/809,900 filed Feb. 25, 2019, and U.S. Ser. No. 62/935,694 filed Nov. 15, 2019, the contents of each are incorporated herein in their entirety.

REFERENCE TO SEQUENCE LISTING

[0002] A Sequence Listing submitted as an ASCII text file via EFS-Web is hereby incorporated by reference in accordance with 35 U.S.C. § 1.52(e). The name of the ASCII text file for the Sequence Listing is 16783383_SequenceListing.txt, the date of creation of the ASCII text file is Jun. 1, 2020, and the size of the ASCII text file is 10 KB.

BACKGROUND

[0003] Organophosphorus (OP) cholinesterase inhibitors, including chemical warfare nerve agents (CWNAs) such as sarin and VX, continue to be a global threat against both military personnel and civilian populations. From conflict zones in third world nations to terrorist attacks in the western world, CWNAs may be employed in a multitude of different ways to wreak havoc on society (FDA, 2018). Inhibition of acetylcholinesterase (AChE) via phosphylation of the active serine site (Marrs, 1993) remains the primary mechanism of toxicity through which CWNAs act. The resulting inhibition results in an accumulation of acetylcholine thereby creating a cholinergic crisis that drives respiratory failure (Giyanwani et al., 2017) which can ultimately be fatal. CWNAs also inhibit butyrylcholinesterase (BChE) without any apparent toxic effects; rather, BChE acts as a bioscavenger that binds circulating CWNAs and removes them from circulation (Golomb, 2008). BChE retains a high amount of structural similarity to AChE (Vellom et al., 1993), and like AChE can also hydrolyze the neurotransmitter acetylcholine. Early animal studies using BChE as a bioscavenging treatment strategy for CWNA poisoning showed long-term resistance to poisoning after intravenous or intramuscular injections of BChE from isolated human serum (Lange et al., 2001). The current standard-of-care for CWNA poisoning includes a three-fold approach, treating those afflicted with muscarinic antagonists (e.g. atropine), AChE reactivators (oximes) (Seidler et al., 1996), and an anticonvulsant (e.g. diazepam) as needed. Despite efforts spanning decades and the creation of numerous oxime compounds, the standard therapy for CWNA poisoning relies on chemicals developed over fifty years ago (Yanagisawa et al., 2006). Additionally, the effectiveness of oxime therapy to treat CWNA poisoning strongly depends on the specific CWNA used as well as the circumstances surrounding exposure. Even so, the dual combination of an atropine-oxime aid via auto-injectors remains the accepted therapy for both civilian and military personnel (Blouin et al., 2016).

[0004] Accordingly, improved compositions and methods for the treatment of OP poisoning are needed. The instant disclosure seeks to address this need in the art.

BRIEF SUMMARY

[0005] Disclosed herein are proteins having at least 90% sequence identity to a wild-type human butyrylcholinesterase and compositions comprising same. The disclosed proteins may have at least one mutation at a position within the acyl binding pocket and at least one mutation adjacent to the acyl biding pocket. Further disclosed are proteins having at least 90% sequence identity to a wild-type human butyrylcholinesterase, wherein the protein may comprise a mutation at a position selected from one or more of 282, 283, and 284.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] This application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0007] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0008] FIG. **1**. Whole blood was obtained from 10 male and 10 female animals per species and processed to red blood cell membranes and plasma as described. Panel A displays AChE activity from the RBC membrane preparations of each individual using acetylthiocholine iodide as the substrate. Panel B displays BChE activity from the plasma preparations of each individual using butyrylthiocholine iodide as the substrate. Each point represents the mean activity from a single sample measured in triplicate in two independent experiments. A two-way Anova with a Tukey's multiple comparisons test was utilized to assess statistical significance. P<0.05-0.01 (*), P<0.001-0.01 (**), P<0.001-0.0001 (***) P<0.0001 (****).

[0009] FIG. **2**. Aging and/or spontaneous reactivation of sarin-inhibited cholinesterases. Activity of sarin-inhibited AChE or BChE in the presence or absence of 2-PAM Cl. Sarin-inhibited RBC membranes from human (A), Yorkshire swine (B) or sarin-inhibited plasma preparations from human (C), Yorkshire swine (D) were incubated at 37° C. until 2-PAM was added and the enzyme activity measured at the times indicated. Data represent average±SEM performed in triplicate. Aging half times were calculated using a non-linear regression model. For simplicity, only the sarin results are displayed above as aging was not observed with VX. Further, only human and one swine model are displayed as all of the primates evaluated yielded similar results and the Gottingen mini pig displayed results similar to the Yorkshire swine. The calculated spontaneous reactivation of plasma BChE following a sarin challenge was 5.0 hours (6.7 hours for the Gottingen mini pig).

[0010] FIG. **3**. Reactivation of VX-inhibited AChE and BChE by 2-PAM Cl. Oxime reactivation of VX-inhibited AChE and BChE was performed as described. Background due to oximolysis was accounted for in each calculation.

[0011] FIG. **4**. Sequence alignment comparison of human BChE vs. porcine (*Sus scrofa*) BChE and sequence of wild type Human BChE Mature Protein Sequence (bottom panel). The red letters indicate differences between the two species. The highlighted region represents the acyl binding pocket of the enzyme. In the bottom panel, amino acid residues 282-285 have been highlighted to identify the location of the mutations of the hybrid enzyme. In the hybrid